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Maternal locoweed exposure *in utero* and as a neonate does not disrupt taste aversion learning in lambs

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ABSTRACT

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Taste aversion learning has been suggested as a sensitive measure of behavioral toxicosis. The purpose of this study was to determine if maternal locowood (*Oxtropis sericea*) ingestion would adversely influence the ability of lambs to acquire and retain a conditioned taste aversion (CTA). Pregnant and lactating ewes were fed locoweed (300 g day^{-1}) from Days 100 to 130 of gestation and/or from Days 10 to 50 of lactation. Surviving lambs were evaluated at 9 months of age. Ingestion of corn (*Zea mays*), a novel feed, was paired with lithium chloride. Lambs were tested for three 15-min periods in a two-choice test (corn and alfalfa pellets) immediately after aversive conditioning; persistence was tested 30 days later using the same procedures. All aversively conditioned lambs formed a strong CTA to corn; previous locoweed history did not influence either the strength or the persistence of the aversion. Even though lambs were overtly intoxicated at birth by maternal locoweed exposure, lambs showed no overt signs of intoxication when this study was conducted. Lambs exposed to locoweed do not have an impaired ability to form taste aversions, suggesting that there were no learning deficits secondary to toxicosis and that lambs are likely to have as normal diet selection patterns as adults.

Keywords: Sheep; Learning; Taste aversion

INTRODUCTION

Oxytropis spp. and *Astragalus* spp. (termed locoweeds in North America) are a serious poisonous plant problem world-wide (James et al., 1981). These plants contain the indolizidine alkaloid, swainsonine, which inhibits cellular glycoprotein processing (Molyneux and James, 1982). Pathological lesions

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from prolonged ingestion are found throughout the gastrointestinal tract and in the brain (Van Kampen and James, 1969, 1970). Affected animals typically have a decreased appetite, locomotor deficits and behave erratically, especially when stressed (James et al., 1981).

Ingestion of locoweed by pregnant ewes can cause fetal malformations and/or abortions, depending on dose and duration (Panter et al., 1987). Nelson et al. (1980) found subtle teratogenic effects in neonatal rats after maternal exposure to locoweed. We recently found that lambs born to ewes consuming about 300 g day^{-1} of locoweed for 30 days in late gestation had locomotor deficits, and were unable to suckle at birth (J.A. Pfister and J.B. Astorga, unpublished data, 1992). Locoweed ingestion may also impair lamb learning (Pfister et al., 1992) and alter food preferences of intoxicated animals (Ralphs et al., 1990).

Conditioned taste aversion (CTA) has been used extensively to study learning processes (Garcia et al., 1985). Animals acquire an aversion to a taste when it is followed by gastrointestinal illness. Garcia et al. (1985) have studied the neuroanatomical pathways that mediate taste aversion learning; such learning is centered in the midbrain and brainstem. Taste aversion learning has been suggested as a sensitive test to determine toxicity (Riley and Tuck, 1985) and has been used to assess learning deficits (Miller and Eckerman, 1986).

The objective of this study was to assess the impact of maternal locoweed ingestion on the ability of lambs to acquire and maintain a taste aversion. Swainsonine is readily passed into milk and nursing animals may develop pathological lesions similar to animals consuming the plant (James and Hartley, 1977), thus we also wished to determine the effect of locoweed ingestion by lactating ewes on nursing lambs. Our hypothesis was that locoweed exposure *in utero* or during lactation would disrupt acquisition and/or persistence of a conditioned taste aversion when animals were tested later in life.

ANIMALS, MATERIALS AND METHODS

Locoweed (*Oxytropis sericea*) was collected fresh from mountain ranges in northern Utah, air-dried at 20°C and ground through a 2-mm screen. Pellets were made by mixing 10% locoweed with alfalfa hay (*Medicago sativa*). Locoweed toxicity is not affected by pelleting (J.A. Pfister, unpublished data, 1991). Locoweed pellets were analyzed for swainsonine concentration by extracting 10 g of material with hot methanol and examination of the extract with gas chromatography (Molyneux et al., 1989).

Estrous was synchronised in 60 nulliparous Targhee×Columbia yearling ewes by implanting them with vaginal pessaries (Internet, Angers, France) containing 40 mg fluorogestone acetate; pessaries were removed after 14 days and ewes were hand-mated 36–48 h later to two fertile rams. Ewes were

scanned using ultrasound at about gestational Day 50; open ewes and ewes carrying multiple fetuses were eliminated from the study. The remaining 27 ewes were divided into two treatments: controls (C, $n=12$) and locoweed (L, $n=15$). Animals in the locoweed treatment were offered 3 kg day^{-1} of the 10% locoweed pellet from Days 100 to 130 of gestation; controls received 3 kg day^{-1} of alfalfa hay.

After parturition, ewe–lamb pairs were further separated into four groups: controls were divided into controls (CC, $n=7$) and locoweed during lactation (Days 10–50) (CL, $n=5$); the original locoweed group was divided into controls (LC, $n=6$) and a locoweed group (LL, $n=6$). Thus, for this study we had lambs that were exposed to locoweed *in utero* (LC), exposed during lactation (CL), exposed during both gestation and lactation (LL), and lambs never exposed to locoweed (CC). Feeders were arranged so that lambs could not consume locoweed pellets when offered to their mothers. Lambs were weaned at about 85 days of age and housed together on alfalfa hay. At the beginning of this study lambs were about 9 months old and weighed about 50 kg. When this study began, experienced observers could not detect any overt residual symptoms from locoweed exposure, even though overt symptoms were readily apparent at birth in the two groups (LC and LL) exposed *in utero* (J.B. Astorga, unpublished data, 1992).

In the aversion trial we added a positive control group (CONT, $n=5$) of similarly aged lambs that were not averted to the stimulus feed. All 29 lambs were individually housed in 2 m$\times$2 m pens in a climate-controlled building at 10°C and fed alfalfa pellets ad libitum. Lambs had been exposed to alfalfa pellets during a study of postnatal development (J.A. Pfister and J.B. Astorga, unpublished data, 1992), thus alfalfa pellets were not a novel feed. Lambs were given 12 days to become habituated to the environment and for feed intake to stabilize. Intake of alfalfa pellets alone was determined over 3 days by fasting lambs for 4 h, then measuring alfalfa pellet consumption during a 15-min test period.

A novel feed, whole corn kernels (*Zea mays*), was used as the stimulus feed in conditioning the taste aversion. Lambs were fasted overnight, then individually offered 30 g of whole corn. After two exposures all lambs, except one on the LL treatment, readily consumed the corn. The deviant lamb accepted corn after six exposures.

Conditioning trials followed the protocol of Burritt and Provenza (1990). Lambs were offered corn alone after a 4-h fast. All lambs consumed 30 g of the stimulus feed and were subsequently dosed with lithium chloride (LiCl) at 200 mg kg^{-1} body weight. This emetic agent was given via gelatin capsules and produces non-lethal gastrointestinal distress (Burritt and Provenza, 1990). This dose of LiCl produced overt symptoms of distress (i.e. reduced activity and feed intake), thus the dosage was reduced to 160 mg kg^{-1} in the next pairing. After a 7-day recovery period, the stimulus feed was offered again

and any lambs ($n=6$) that consumed 30 g of corn were immediately dosed with LiCl capsules.

Immediately after the pairing of LiCl with the stimulus feed, all lambs were tested for aversion learning over 3 days by offering lambs both corn and alfalfa pellets for 15 min after a 4-h fast. We limited the maximum amount of corn that could be consumed during a test to 100 g to avoid potential acidosis from excess corn ingestion.

Persistence of the aversion was determined 30 days after the initial tests for aversion learning. Lambs were given 2 days to adapt to being penned individually; corn and alfalfa pellets were offered for 15 min as described previously.

The experimental design was a completely random design with repeated measures over time. The 3-day alfalfa intake test, the 3-day two-choice test and the 3-day persistence test were compared using the ANOVA procedures of the Statistical Analysis Systems Institute (SAS) (1987). Significant F-tests ($P<0.05$) were followed by the Least Significant Difference (LSD) procedure to separate means.

RESULTS

The 10% locoweed pellets contained 0.0011% swainsonine, which is comparable with leaf and stem material used in other studies (Molyneux and

TABLE 1

Mean corn consumption (percentage of amount offered) during conditioning when corn consumption was paired with LiCl dose, and the number of subjects consuming 100% of offered corn at that pairing[1]

Treatment groups[2]		Pairings						
		First			Second			
		Corn eaten (%)	Number eating 100%	Number eating 0%	Corn eaten (%)	Number receiving a 2nd LiCl dose	Number eating 100%	Number eating 0%
CONT	($n=5$)	100	5	0	100	–	5	0
CC	($n=7$)	100	7	0	18	2	0	5
CL	($n=5$)	100	5	0	17	2	0	3
LC	($n=6$)	100	6	0	18	1	1	5
LL	($n=6$)	100	6	0	20	1	1	5

[1]After initial exposure to corn, a novel feed, subjects were offered 100 g of corn and dosed with LiCl if they consumed ≥ 30 g of corn. At the second pairing, subjects were again offered 100 g of corn and dosed with LiCl if they consumed ≥ 30 g of corn.

[2]CONT, subjects not exposed to locoweed, and not conditioned with LiCl; CC, subjects not exposed to locoweed, but aversively conditioned with LiCl; CL, subjects exposed to locoweed only during lactation and aversively conditioned with LiCl; LC, subjects exposed to locoweed only *in utero*, and aversively conditioned with LiCl; LL, subjects exposed to locoweed both *in utero* and during lactation and aversively conditioned with LiCl.

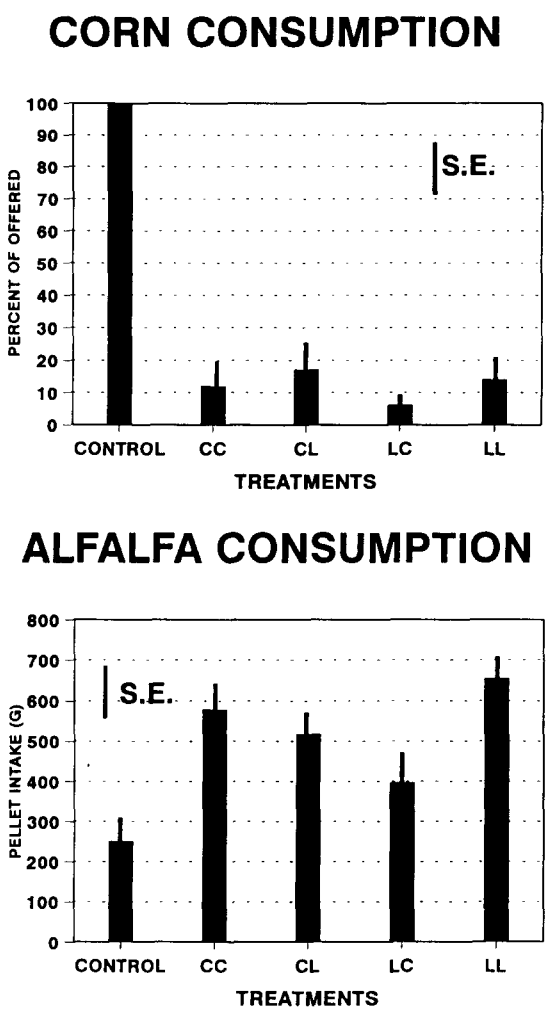

Fig. 1. Mean consumption of whole corn (per cent of corn offered) and alfalfa pellets (g) by lambs in various treatments during three, 15-min two-choice tests to measure taste aversion learning. Control lambs were not aversively conditioned; lambs in all other treatments were conditioned using LiCl capsules. There is no error bar for the Control corn group because all animals ate all of the corn each day.

James, 1982). Three ewes on the locoweed treatment aborted and were eliminated from the study.

Most lambs were averted after just one pairing of corn with LiCl (Table 1). Six of the 24 treated lambs required a second LiCl dose.

No treatment differences ($P > 0.05$) were noted for the three, 15-min alfalfa pellet intake trials; there was no treatment–day interaction and no day effect ($P > 0.05$) (data not shown).

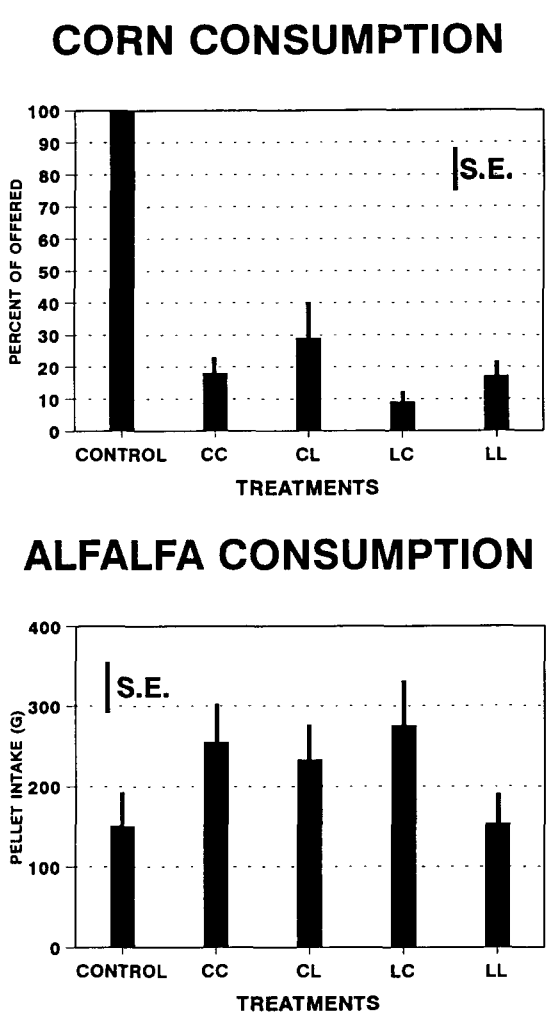

Fig. 2. Mean consumption of corn (per cent of corn offered) and alfalfa pellets (g) by lambs in various treatments during three, 15-min two-choice tests to measure persistence of taste aversion learning. Persistence was measured 30 days after the initial tests.

The unaverted CONT group differed ($P<0.001$) from the CC, CL, LC and LL groups in corn intake during the 3 days of testing for an aversion, indicating the preference of the unaverted lambs and the aversion of the averted animals for corn. The averted groups ate 11% of the corn offered during the 15-min period, while the CONT group consumed 100% of the corn (Fig. 1). Conversely, the CONT group consumed fewer alfalfa pellets ($P<0.002$) than did the CC, LL and CL groups during the 15-min trial periods (Fig. 1).

The CONT group consumed more corn and less alfalfa pellets ($P<0.01$) during the persistence tests than all other groups (Fig. 2). In the two-choice tests, all the averted groups tended to consume more corn each day, as indicated by day effects (corn: $P=0.05$; alfalfa: $P<0.01$). There was a treatment–day interaction for alfalfa intake, but not for corn consumption.

DISCUSSION

Maternal locoweed ingestion during gestation and/or lactation had no effect on taste aversion learning or persistence of an aversion in 9-month-old lambs. There are three possible reasons why locoweed failed to disrupt taste aversion learning: (1) even though lambs exposed to locoweed *in utero* were severely affected at birth and some lambs may show overt symptoms of neurotoxicity for several weeks after birth (J.A. Pfister and T.A. Weber, unpublished observations, 1992), residual neurotoxic effects from locoweed may have regressed in the study lambs (James and Van Kampen, 1971; Nelson et al., 1980); (2) residual lesions may have had no impact on the portions of the brain associated with taste aversion learning; (3) even though the taste aversion paradigm has been suggested as a sensitive means to evaluate toxicity and learning deficits (Riley and Tuck, 1985; Miller and Eckerman, 1986), the CTA paradigm may not be sensitive enough to detect some types of residual neurotoxicity.

Fetal exposure to locoweed during gestation produces all lesions in the fetus that are exhibited by the dams (James, 1972; Hartley and James, 1973; James, 1976). There are indications that fetal lesions partially regress once the toxic insult is removed, but there may be permanent neurological injury to the developing fetus owing to maternal exposure (Hartley and James, 1973; Nelson et al., 1980). In adult animals, even though pathological lesions may regress, some residual neuronal damage is not repaired and apparently recovered animals retain signs of intoxication (James and Van Kampen, 1971). We are continuing to examine these lambs for behavioral toxicology, thus no pathology results are available at present.

Neural mechanisms for taste aversion learning have been described (Garcia et al., 1985) and are centered in the brainstem and midbrain (Garcia y Robertson and Garcia, 1987). The pathology of locoweed is incomplete as to dose-duration effects, but evidence to date indicates that swainsonine affects nerve cell bodies in the central nervous system (Van Kampen and James, 1969, 1970; Hartley et al., 1989). Brain lesions in such areas as the thalamus (Van Kampen and James, 1969) could disrupt taste aversion learning, since the thalamus is a key structure for taste (Kandel and Schwartz, 1985). Locoweed lesions are numerous in Purkinje cells in the cerebellum (Hartley et al., 1989) and cerebellar lesions may disrupt classical conditioning and discrimination learning (Lalonde and Botez, 1990).

The dose of swainsonine consumed by sheep exposed to locoweed has seldom been quantified. Assuming complete transfer of swainsonine to fetal tissues (James et al., 1989), lambs *in utero* received about 33 mg day^{-1} of swainsonine for 30 days; suckling lambs received an unknown amount of swainsonine via the mother's milk. The ewes showed characteristics of mild to moderate intoxication, while lambs showed overt signs of intoxication that apparently did not persist beyond the first few weeks after birth.

CONCLUSIONS

Grazing livestock often ingest locoweed during gestation and/or lactation. Our results indicate that offspring that survive the toxic insult *in utero* or from the mother's milk will show no impairment of CTA learning. Livestock producers that have sheep consuming locoweed for a similar duration during gestation or lactation can retain the offspring with little fear of residual impairment of food aversion learning. The results suggest that there were no persistent learning deficits secondary to toxicosis and that lambs are likely to have normal diet selection patterns as adults (Ralphs et al., 1991).

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